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Chronic Infection with Hepatitis and Herpes Viruses in Patients with Sjogren's Disease

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The prevalence of hepatites B, C, E, and G viruses, Epstein—Barr virus, and type 6 herpesvirus was studied in Russian and Norwegian patients with Sjogren's disease. The incidence of HBV, HCV, HEV, and HGV markers in Russian patients was higher than in donors. The incidence of serological markers of Epstein—Barr and type 6 herpesvirus was virtually the same in the patients with Sjogren's disease and donors. Epstein—Barr virus DNA was less frequently detected in patients with Sjogren's disease than in donors, as was shown by blood and salivary DNA testing.

Key Words: *Sjogren's disease; hepatitis and herpes viruses; polymerase chain reaction; enzyme immunoassay and immunofluorescent analysis*

Sjogren's disease is a systemic autoimmune rheumatic and lymphoproliferative disease characterized by involvement of the salivary, lacrimal, and other exocrine glands. Involvement of the salivary and lacrimal glands in the presence of another rheumatic disease is diagnosed as Sjogren's syndrome.

The etiology of autoimmune diseases, including Sjogren's disease, is unknown and can be associated with a complex of genetic, immune, hormonal, and infectious factors.

Viruses can induce an autoimmune response in Sjogren's disease. Herpesviruses (Epstein—Barr virus, EBV; type 6 herpesvirus, HHV-6), hepatites B (HBV) and C (HCV) viruses, *etc.* are now considered to be potential triggering factors in the etiology of Sjogren's disease [10].

Salivary glands, the main target organ in Sjogren's disease, simultaneously act as a store of latent viral infection with EBV [7,12], HCV [5,8], HHV-6 [2], and HBV [1]. It was recently demonstrated that hepatitis G virus (HGV) induces acute and chronic forms of infection. The incidence of this virus in patients with autoimmune diseases is little studied. According to J. Font *et al.* [4], the incidence of HGV infection is not increased in patients with Sjogren's disease in comparison with healthy population of the same geographical region.

Lymphocytic infiltration of exocrine glands and high production of autoantibodies to ribonucleoproteins Ro/SS-A and La/SS-B are observed in patients with Sjogren's disease. Anti-DNA antibodies are also sometimes detected in patients with Sjogren's disease and Sjogren's syndrome [11]. A case with Sjogren's disease with pronounced systemic manifestations and high titers of antinuclear antibodies after primary EBV infection (infectious mononucleosis) was reported [6].

We investigated chronic infection with HBV, HCV, HEV, HGV, EBV, and HHV-6 and measured anti-DNA antibodies in patients with Sjogren's disease.

MATERIALS AND METHODS

Several groups of patients with Sjogren's disease and syndrome were examined. The reference group

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TABLE 1. Groups of Examinees

Group	Men/women; age, years	Place of testing	Material from
Patients with Sjogren's disease (*n*=23)	0/23; 19-74	1, 3	2
Patients with chronic hepatitis(*n*=24)	14/10; 16-66	1	2
Patients with chronic hepatitis and Sjogren's disease (*n*=23)	2/22; 16-65	3	2
Primary blood donors (*n*=709)	417/292; 16-58	1	1
Patients with Sjogren's disease (*n*=150)	9/141; 27-83	4	5
Primary blood donors (*n*=150)	86/64; 18-57	4	5

Note. *1*) N. F. Gamaleya Institute of Epidemiology and Microbiology, Moscow; *2*) E. M. Tareev Clinic of Therapy and Occupational Diseases, Moscow; *3*) Institute of Infectious Diseases Control, Stockholm; *4*) Brogelmann University Laboratory, Bergen; *5*) Rheumatology Clinic, Bergen.

consisted of primary blood donors (Table 1). Sjogren's disease was diagnosed in accordance with classification criteria by minor salivary gland biopsy from the lower lip and by counting lymphocyte infiltration foci containing more than 50 lymphocytes per 4 mm^2 surface in a salivary gland biopsy specimen (Focus score). The serum was assayed for the presence of rheumatoid factor, antibodies to ribonucleoproteins Ro/SS-A and La/SS-B, and antinuclear antibodies.

Markers of viral infection in the blood and saliva were detected by PCR, enzyme immunoassay, and immunofluorescent analysis.

Polymerase chain reaction (nested PCR) was carried out with two primers (external and internal) to EBV genome site encoding type 2 nuclear antigen (EBNA-2, Table 2). PCR (common variant) for detecting EBV DNA in the saliva was carried out with primers to EBV genome sites encoding EBV RNA (EBER-1) and EBV proteins (EBNA-1, type 1 latent membrane protein, LMP-1, and capsid glycoprotein GP350/220). HGV in patients and donors was also detected by PCR (common variant).

TABLE 2. Primers for Detecting EBV DNA by PCR

Infection marker	Sequence (5'-3')	Length of DNA site, b. p.
EBNA-1	GTCATCATCATCCGGGTCTC	269
	TTCGGGTTGGAACCTCCTTG	
EBNA-2	ATCAATGCACCCTCTTACTC	352
	GCTCTGGTCTCCAAGGTCCA	
	ACCACGGTCCCCGACTGTAT	198
	CGGCTCTGGCCTTGAGTCTT	
LMP-1	ACTCTGCTCTCAAAACCTAGGC	259
	ATTTCCAGCAGAGTCGCTAG	
EBER-1	AGGACCTACGCTGCCCTAGA	167
	AAAACATGCGGACCACCAGC	
GP350/220	GCACGCCCCCCCAAAATGCA	482
	TTATACATAGGTCTCGGCGT	

The reaction mixture for PCR (final volume 25 μl) contained: 0.1 μl analyzed DNA, 2.5 μl PCR buffer (PCR Optimization Kit, Fermentas), 2 mM dNTP, 0.5 μl each primer, and 1.25 U Taq polymerase (5 U/liter, Fermentas).

Enzyme immunoassay was used for detecting markers of infection with HBV, HCV, HEV, EBV viruses and anti-DNA antibodies. Standard kits AxSYM, HBsAg, AxSYM HCV 3.0 (Abbott Laboratories), EBV IgG ELISA (Gull Laboratories) were used.

Immunofluorescent analysis for detecting markers of EBV and HHV-6 infection was carried out using standard Biotrin International kit (Cedar Knolls).

The significance of differences was evaluated using χ^2 test.

RESULTS

Surface antigen (HBsAg), the main marker of HBV, was detected in 3 (25%) of 12 Russian patients with Sjogren's disease and 18 (2.5%) of 709 primary donors. The total incidence of anti-HBs and anti-HBc antibodies was 75% (9 patients with Sjogren's disease). HBe antigen was not detected in patients with Sjogren's disease. Antibodies to HCV were detected in 4 (33.33%) of 12 patients with Sjogren's disease and 9 (1.27%) of 709 donors.

In Norwegians, there was no difference between patients with Sjogren's disease and controls. HBsAg was detected in only 1 (2.04%) of 49 patients. No one examine had antibodies to HCV. No markers of hepatitis virus infection were detected in controls.

In Russians, HGV RNA was detected in 2 (16.67%) of 12 patients with Sjogren's disease, 4 (16.67%) of 24 patients with chronic hepatites B and C, and 2 (4%) of 50 donors.

Antibodies to HEV were detected in 2 (16.67%) of 12 patients with Sjogren's disease and 9 (1.45%) of 134 donors. These results indicate a higher per-

TABLE 3. Incidence of Anti-DNA Antibodies (Titer 1:80) in Patients with Sjogren's Disease

Antibodies	Sjogren's disease (n=11)		Sjogren's syndrome (n=23)	
	abs.	%	abs.	%
IgGAM	8	72.7	8	34.8***
IgG3	8	72.7	5	21.7*
IgM	6	54.5	3	13.0**

Note. *p<0.005, **p<0.01, ***p<0.025 *vs.* patients with Sjogren's disease.

centage of HEV-seropositive subjects among patients with Sjogren's disease in comparison with healthy population of the region.

High titers of antibodies to capsid and early EBV antigens were detected in 3 (37.5%) of 8 Russian patients with Sjogren's disease and high titers of antibodies to HHV-6 in 1 patient, which indicates active replication of these viruses in some of patients with Sjogren's disease. Serological profile of one patient corresponded to typical picture of nasopharyngeal cancer, despite the absence of clinical manifestations.

In Norwegians, antibodies to EBV capsid antigen were found in 95 (94.06%) of 101 patients with Sjogren's disease and 92 (92.0%) of 100 donors.

DNA encoding EBNA-1 was detected in 40 (56.34%) of 71 patients with Sjogren's disease and 55 (74.32%) of 74 donors. No association between the presence of EBV DNA and laboratory findings was observed (Table 3).

EBV DNA encoding EBNA-2 was detected in DNA isolated from the saliva in 9 (47.5%) of 19 patients with Sjogren's disease and in 11 (78.57%) of 14 primary blood donors. EBV DNA encoding viral EBNA-1 RNA was found in DNA isolated from the saliva of 3 (15.8%) of 19 patients and in none of the donors. EBV DNA encoding other EBV antigens was not detected either in the patients or blood donors.

Antibodies to HHV-6 were detected in 16 (84.21%) of 19 patients and 15 (78.95%) of 19 blood donors.

Detection of anti-DNA antibodies showed statistically significant difference between patients with Sjogren's disease and syndrome (Table 3).

HEV does not cause chronic hepatitis. We detected antibodies to HEV in 2 patients, which is comparable with the number of patients with markers of HBV, HCV, and HGV infection. Further studies of HEV markers on a larger group of patients are needed.

In contrast to the data obtained by J. Font *et al.* [4], our results indicate high incidence of HGV RNA in patients with Sjogren's disease, which indicates possible participation of HGV as a trigger or virus cofactor in the mechanisms of pathogenesis of both autoimmune hepatitis and Sjogren's disease and deserves further investigation.

Serological screening for EBV markers showed no statistically significant difference in the levels of positive results in Norwegian patients with Sjogren's disease and donors.

A high level of EBV infection in the main population [3] complicates evaluation of the prevalence of EBV infection in Norwegian patients with Sjogren's disease. PCR detected EBV DNA in the blood of Norwegian patients with Sjogren's disease and donors. The results correlate with the data obtained by M. A. Oosterveer *et al.* [9] who detected EBV DNA in the blood of 10 (61%) of 18 patients with Sjogren's disease and 9 (75%) of 12 blood donors.

EBV DNA encoding EBNA-2 was also detected in the saliva of Norwegian patients with Sjogren's disease and donors. These data probably indicate active release of EBV to the oral cavity as a result of virus reactivation in the salivary glands.

Oropharyngeal and salivary gland epithelium is a possible reservoir of EBV. The development of Sjogren's disease can result from immune recognition of viral antigens in salivary glands, leading to destruction of salivary glands. EBV, an inductor of polyclonal activation of B lymphocytes, can induce the production of antibodies with a wide spectrum of specificity, which can induce a systemic autoimmune process. Chronic infection of the salivary glands can serve as an endogenous source of the virus and lead to secondary infection of B lymphocytes. Reactivation of EBV resultant from impaired immune response or other potential factors (coinfection with other herpesviruses or HCV) in patients with Sjogren's disease is a possible cause of EBV involvement in the development of this disease.

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